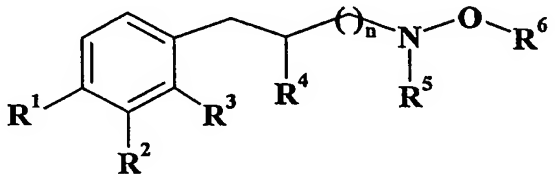
HYDROXYLAMINE DERIVATIVES

This invention is related to hydroxylamino derivatives of the following general formula (I)



(I)

wherein

n is 0, 1 or 2;

R^1 and R^2, independently of each other, are H, OH or OCH_3;

R^3 is H or CH_3;

R^4 is H, C_1-C_3 straight or branched alkyl or, together with R^3, forms a five to seven-membered carbocyclic ring;

and R^5 and R^6, independently of each other, are H or C_1-C_5 straight or branched alkyl

and the pharmaceutically acceptable salts or prodrug thereof, for the preparation of medicaments useful for the prevention, treatment and diagnosis of central and peripheral degenerative disorders related to protein misfolding and/or misaggregation.

The invention also relates to novel compounds included in the above formula (I), to a method for preparing said compounds and to pharmaceutical compositions containing them.

FIELD OF INVENTION

The present invention relates to novel compounds, pharmaceutical compositions containing said compounds and their use in the treatment and diagnosis of central and peripheral nervous system degenerative disorders such as those caused by formation of fibrils of beta-amyloid peptide,

alpha-synuclein, prion protein and huntingtin, Alzheimer's Disease, Lewy body disease, Parkinson's Disease, spongiform encephalopathies, Huntington's Disease and systemic AA amyloidosis including the primary amyloidosis of the peripheral nervous system.

BACKGROUND OF THE INVENTION

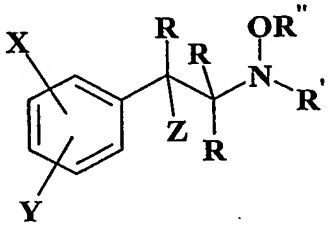
In recent years it has been found that several neurodegenerative disorders are caused by protein misfolding and/or misaggregation.

One of the most important and initial step of Alzheimer's disease (AD), for instance, involves proteolytic cleavage of APP (amyloid precursor protein,) releasing short 40, 42 and 43 aa peptides (beta amyloid 1-40, 1-42, and 1-43). The degeneration of neurons is due to polymerization of beta-amyloid peptides (Aβ) and subsequent neuronal deposit (amyloid). Monomeric Aβ is a product of normal metabolism and is not toxic to neuronal cells. As it forms multimeric and polymeric assemblies of itself, Aβ acquires potent toxicity for neuronal cells. Inhibition of this polymerization process has thus been identified as a potential approach to the treatment of AD and all other related pathologies where the anatomopathological hallmark is the presence of Aβ deposit.

Amyloid like-disorders might be far more widespread than previously thought, and might include many common neurodegenerative and neuromuscular pathologies, as well as prion disease. Prion diseases can be either sporadic or infectious, and until recently were not known to be associated with protein misfolding and deposition. Prions are composed solely of a misfolded prion protein (PrP^{Sc}) isoform of a glicolipid-anchored host protein. Patients with prion diseases develop progressive neurologic dysfunction. Prion diseases are invariably fatal and no effective therapy exists till now. Compounds that inhibit PrP^{Sc} formation including Congo red, are effective in scrapie-infected cultured cells.

It has also been found that the formation of intraneuronal deposits called Lewy bodies and Lewy neurites is due to aggregates of another protein, alpha-synuclein, whose misfolding and misaggregation is also believed to be one of the causes of both AD and Parkinson's disease.

US 3,184,510 discloses N-alkoxy and N-hydroxyphenylethylamines of the following general formula:



wherein

X and Y, independently of each other, are H, OH or OCH_3;

Z is H or OH;

R is H or CH_3;

R' is H, CH_3, C_2H_5, C_3H_7 or *i*-C_3H_7;

R" is CH_3, C_2H_5, C_3H_7 or *i*-C_3H_7

and their use for sustaining and/or raising blood pressure, their use as local vasoconstrictors and/or in the relaxation of the bronchial smooth muscles and of the intestinal tract, in pupil dilation and in the stimulation of adrenergic nerves. No CNS activity was disclosed.

GB 1,062,299 discloses 3,4-dihydroxyphenyl-propane derivatives of the general formula Ar-CH_2-C(CH_3)-NH(OR), wherein Ar is 3,4-dihydroxyphenyl and R is H or C_1-C_8 alkyl, as hypertensive agents.

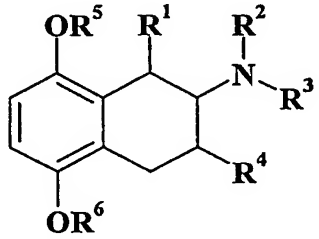
Major, R. T. and Ohly, K. W. J. (Med. and Pharmaceut. Chem. 1961, 4, 51-65) described the synthesis of N-alkoxy-N-(2-phenyl)-isopropylamines of formula $C_6H_5CH_2CH(CH_3)NHOR$ wherein R is CH_3, C_2H_5 or *i*-C_3H_7, and tested the compounds for MAO inhibitory activity.

Benington, F.; Morin, R. D. and Clark, L. C. Jr. (J. Med. Chem. 1965, 8, 100-104) described the synthesis of ring-substituted 1-aryl-2-hydroxyamino- and 1-aryl-2-methoxyamino-propanes and demonstrated that the compounds were general central stimulants.

Kende *et al.* described in Tetrahedron Letters 1991, 14, 1699-1702 the synthesis of hydroxylamino derivatives using samarium diiodide as reducing agent.

None of the above mentioned documents mentions the use of the compounds as inhibitors of protein and/or peptide fibrils aggregation.

WO99/62505 describes a method for the treatment of a neurodegenerative disorder comprising the administration of compounds able to inhibit the binding of an amyloid beta peptide to alpha-7 nicotinic acetylcholine receptors. This patent application claims compounds of the general formula:

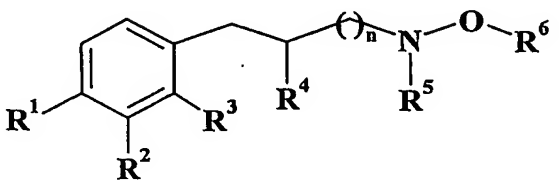


wherein R^2 is selected from hydrogen, C_1-C_6 alkyl, aryl or C_7-C_{10} aralkyl and R^3 is selected from hydrogen, C_1-C_6 alkyl or C_3-C_{10} alkenyl.

WO 01/30979 discloses pharmaceutical compositions comprising primary N-hydroxylamines of the general formula $NHOHCR_1R_2R_3$, wherein R_1, R_2 and R_3 are independently selected from hydrogen, substituted or unsubstituted (C_1-C_{10}) alkyl, alkenyl, alkynyl, aryl, acyl, carboxyl, amino, nitro, nitroso, oxime, hydrazone, azo, thiol, sulfonyl and halide and their use for reducing oxidative damage.

DESCRIPTION OF THE INVENTION

The present invention relates to the use of compounds of formula (I)



(I)

pharmaceutically acceptable salts or prodrugs thereof, wherein:

n is 0, 1 or 2;

R^1 and R^2, independently of each other, are H, OH or OCH_3;

R^3 is H or CH_3;

R^4 is H, C_1-C_3 straight or branched alkyl or, together with R^3, forms a five to seven-membered carbocyclic ring;

and R^5 and R^6, independently of each other, are H or C_1-C_5 straight or branched alkyl

for the preparation of pharmaceutical compositions for the prevention, treatment, diagnosis of central and peripheral nervous system disorders involving protein misfolding and/or misaggregation, for example disorders caused by formation of fibrils of beta-amyloid peptide, alpha-synuclein, prion protein and huntingtin, such as Alzheimer's Disease, Lewy body disease, Parkinson's Disease, spongiform encephalopathies, Huntington's Disease and systemic AA amyloidosis including the primary amyloidosis of the peripheral nervous system.

The invention also relates to compounds of formula (I) as defined above and pharmaceutically acceptable salts thereof

with the provisos that:

R^1 and R^2 cannot be both hydrogen;

when n is 0, R^1 and R^2 are both hydroxyl, R^3 and R^5 are hydrogen, R^4

cannot be CH_3 (GB 1,062,299);

when n is 0, R^3 is H and R^4 is H or CH_3, R^6 cannot be C_1-C_3 straight or branched alkyl (U.S. 3,184,510);

and that the compounds cannot be:

- 1-(4-hydroxyphenyl)-2-hydroxylaminoethane, (J. Biol. Chem. 1979, 254, 8575-8583);
- 1-(4-hydroxyphenyl)-2-hydroxylaminopropane, (J. Pharm. Pharmac. 1973, 25, 708-717);
- 1-(4-methoxyphenyl)-2-hydroxylaminopropane, (J. Med. Chem. 1965, 8, 100-104, J. Pharm. Pharmac. 1973, 25, 708-717);
- 1-(3,4-dimethoxyphenyl)-2-hydroxylaminopropane, (J. Med. Chem. 1965, 8, 100-104, Tetrahedron 1975, 31, 1531-1535);
- 1-(4-methoxyphenyl)-4-hydroxylaminobutane, (Tetrahedron Letters 1991, 32, 1699-1702);
- 1-(3-methoxyphenyl)-2-hydroxylaminopropane, (Chem. Pharm. Bull. 1981, 29, 1615);
- 1-(3,4-dimethoxyphenyl)-2-hydroxylaminoethane, (WO92/00968);
- N-methyl-1-(3,4-dihydroxyphenyl)-2-hydroxylaminopropane, (Xenobiotica 2003, 33, 1013);
- 1-(3-methoxy-4-hydroxyphenyl)-2-hydroxylaminopropane, (Xenobiotica 2003, 33, 1013);
- N-methyl-1-(3-methoxy-4-hydroxyphenyl)-2-hydroxylaminopropane, (Xenobiotica 2003, 33, 1013);
- N-methyl-1-(3,4-dimethoxyphenyl)-2-hydroxylaminopropane, (Tetrahedron 1975, 31, 2595).

Preferred novel compounds are:

- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;

- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-butyl-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-butyl-O-propyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-methyl-

hydroxylamine;

- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-methyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;

- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-methyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-propyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-methyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-propyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-propyl-O-ethyl-hydroxylamine.

Preferred known compounds for the use of the invention are:

- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-O-methyl-hydroxylamine;

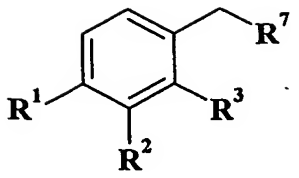
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;

The present invention includes all the possible optical isomers of the compounds of formula (**I**) and their mixtures, as well as their metabolites. Some crystalline forms of the compounds may exist as polymorphs, which are also included in the present invention. Some of the compounds are solvated with water, and as such they are also intended to be encompassed within the scope of the invention. The invention also includes pharmaceutically acceptable bioprecursors and prodrugs of compounds of formula (**I**). Selection and preparation of prodrugs are described, for example, in "Design of Prodrugs", ed. H. Bundgaard, Elsevier, 1985.

Suitable pharmaceutically acceptable salts of compounds of formula (**I**) include acid addition salts with inorganic acids, e.g. nitric, hydrochloric, carbonic, hydrobromic, sulphuric and phosphoric acid, or with organic acids, e.g. acetic, propionic, glycolic, lactic, oxalic, malonic, succinic, maleic, fumaric, tartaric, citric, benzoic, cinnamic, mandelic, methanesulphonic, salicylic acid.

The compounds of the invention can be prepared by different methods.

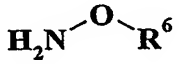
According to a first method, a compounds of formula (**II**)



(II)

wherein R^1, R^2, R^3 are as defined above and R^7 is $-C(=O)R^4$, $-CH(R^4)-CHO$, or $-CH(R^4)-CH_2-CHO$, wherein R_4 is as defined above

is reacted with a compound of formula **(III)**



(III)

wherein R^6 is as defined above,

in the presence of a reducing agent to give a compound of formula **(I)** wherein R^5 is hydrogen. This is subsequently alkylated with a compound of formula **(IV)**:

$$R^5—X$$

(IV)

wherein R^5 is C_1-C_5 straight or branched alkyl and X is a halogen atom or a leaving group, preferably selected from mesylate, tosylate or triflate.

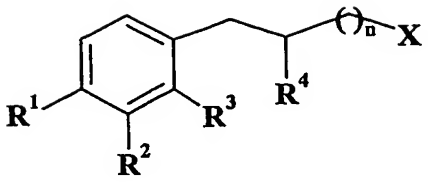
Alternatively, compounds of formula **(I)** wherein R^5 is hydrogen can be subjected to reductive alkylation with a compound of formula **(V)**:

$$R^8\text{-}CHO$$

(V)

wherein R^8 is hydrogen or C_1-C_4 alkyl.

Compounds of formula **(I)** wherein R^5 is hydrogen can also be obtained by reacting a compound of formula **(VI)**



(VI)

wherein n, R^1, R^2, R^3, R^4 and X are as defined above

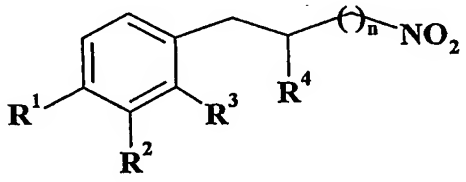
with a compound of formula (VII)

$R^6ONHCOOC_2H_5$

(VII)

wherein R^6 is as defined above in the presence of a base and subsequent hydrolysis of the resulting carbamate.

According to a further method, a compound of formula (VIII)

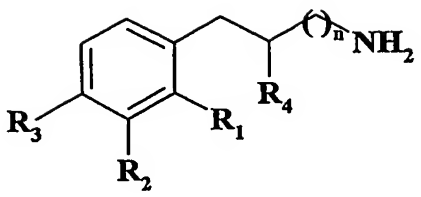


(VIII)

wherein n, R^1, R^2, R^3 and R^4 are as defined above,

is reduced with BH_3·THF, $NaBH_4$, Zn/NH_4Cl, SmI_2 (Kende, A.S. and Mendoza, J. S. *Tetrahedron Letters* 1991, 32, 1699-1702), to give compounds of formula (I) where both R_5 and R_6 are hydrogen. N- and/or O-alkylation can be performed according to methods described in the literature and well known to those skilled in the art.

The compounds of the general formula (I) wherein both R^5 and R^6 are hydrogen can also be obtained by alkylation of the amino group of compounds of general formula (IX)



(IX)

wherein n, R^1, R^2, R^3 and R^4 are as defined above

with YCH_2CN (with Y = Cl, Br, I), oxidation with m-CPBA, and subsequent hydrolysis with hydroxylamine (H. Tokuyama *et al. Synthesis* 2000, 9, 1299-1304).

Compounds (II), (III), (IV), (V), (VI), (VII), (VIII) and (IX) are commercially available or can be prepared from commercially available compounds by conventional methods.

Reductive amination is preferably performed under nitrogen atmosphere, in a suitable organic solvent, preferably an alcohol, at a temperature ranging from about 0°C to about 40°C. The reduction can be carried out with hydrides, preferably selected from $NaBH_4$, $NaBH_3CN$ or by catalytic hydrogenation, the most appropriate catalyst being PtO_2. Molecular sieves can optionally be added to the reaction mixture to promote the reaction.

The reaction of compounds of formula (VI) with compounds of formula (VII) is carried out in alkaline conditions, in solvents like alcohols, THF, acetonitrile, at temperatures ranging from room temperature to 100°C.

In compounds of the general formulas (IV) and (VI), X is preferably iodine or mesylate and alkylation can be carried out in a suitable organic solvent, preferably selected from methanol, ethanol or isopropanol, more preferably ethanol, at a temperature ranging from about 0°C to about 50°C.

The reductive alkylation of compounds of formula (I) wherein R_5 is hydrogen with an aldehyde of formula (V) can be carried out in a suitable organic solvent, such as an alcohol, e.g. methanol, ethanol or acetonitrile in the presence of a suitable reducing agent, such as sodium cyanoborohydride,

at a temperature ranging from about 0°C to about 30°C.

The reduction of the nitro group of compounds of the general formula (VIII) to hydroxylamino group can be carried out according to conventional methods, preferably under nitrogen atmosphere with diborane or $NaBH_4$ in THF at a temperature ranging from about 0°C to about 25°C, or with SmI_2 in THF/methanol at room temperature.

The oxidation of compounds of the general formula (IX) can be carried out according to Tokuyama, H. et al. Compounds of the general formula (IX) are first treated with Y-CH_2CN, in a suitable organic solvent, preferably acetonitrile or DMF, with a suitable base, preferably Hünig's base (N,N-diisopropylethylamine) or K_2CO_3 and subsequently oxidised with m-CPBA in a suitable organic solvent, preferably CH_2Cl_2, at a temperature ranging from room temperature to 40°C; the final treatment with hydroxylamine is carried out in an alcoholic solvent, preferably in boiling methanol.

PHARMACOLOGY

The compounds of the invention are able to interfere with the in vitro aggregation, fibrilization and deposition of different types of self-aggregating proteins, such as Amyloid-β_{1-42}, Prion $Protein_{106-126}$ and α-synuclein.

In our experimental conditions, the peptide monomer (anti-aggregation protocol) or already aggregated (disaggregation protocol) was incubated at 37°C, alone or in the presence of the test compound, for different time intervals, then centrifuged and both the supernatant and the pellet were analyzed by HPLC or Thioflavine T binding assay.

The potencies of the compounds of this invention in inhibiting the aggregation or in inducing the disaggregation of the fibrils are in low μMolar range and at least in 1:10 molar ratio to the peptide concentration.

As shown in Table 1, the compound N-(1-methyl-2-(3,4-dihydroxy-

phenyl)-ethyl)-O-methyl-hydroxylamine **(1)** and the compound N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphtalene-2-yl)-N-propyl-O-ethyl-hydroxylamine **(2)** display significant anti-aggregating properties against the all three proteins tested ($A\beta_{1-42}$, $PrP_{106-126}$ and α-synuclein). As compared to the known compound trans-N-(5,8-hydroxy-3-methyl-1,2,3,4-tetrahydro-naphtalene-2yl)-N,N-dipropyl-amine **(3)**, described in WO99/62505 and Bioorg.Med.Chem. 10 (2002) 3565-3569, compound **(2)** is significantly more potent in inhibiting the aggregation of all three proteins, whears compound **(1)** is more potent in inhibiting the aggregation of $A\beta_{1-42}$ and α-synuclein and equally potent in inhibiting $PrP_{106-126}$ fibrils.

Table 1: In vitro Amyloid-β_{1-42}, Prion Protein$_{106-126}$ and α-synuclein fibril formation

Compound	β-Amyloid $_{1-42}$	$PrP_{106-126}$	α-synuclein
	Anti-aggregation (HPLC assay) IC_{50}^{*}, μM	Anti-aggregation (HPLC assay) IC_{50}, μM	Anti-aggregation (HPLC assay) IC_{50}, μM
1	15	76	222
2	6	20	35
3	60	75	878

* IC_{50} = Concentration able to inhibit the aggregation of the fibrils by 50%

Pharmaceutical compositions of compounds of formula **(I)** for oral, parenteral, rectal, sublingual, intranasal or transdermal administration can be prepared according to conventional methods and with conventional excipients or carriers, for example as disclosed in Remington's Pharmaceutical Sciences Handbook, XVII ed., Mack Pub., N.Y., U.S.A.. The effective dose ranges from 0.1 mg/Kg and 100 mg/Kg. Optimal dosages may be determined by those

skilled in the art, and will vary according to the compound, the administration route and the development of the disease. Patient-associated parameters, such as body weight, age, sex, diet, physical activity, period of administration, associated co-morbidities and clinical conditions will also be taken into account.

Preferred pharmaceutical compositions for oral administration are preferably tablets, sublingual tablets, compressed or coated pills, dragees, sachets, hard or soft gelatine capsules. Suitable excipients or carriers include diluents, preferably lactose, dextrose, sucrose, mannitol, sorbitol, cellulose; lubricants, preferably silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binders, preferably starches, gelatine, methylcellulose, carboxymethylcellulose, arabic gum, tragacanth, polyvinylpyrrolidone; disgregants, preferably starches, alginic acid, alginates, sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, preferably lecithin, polysorbates, laurylsulphates; and, in general, non-toxic.

Liquid dispersions for oral administration are preferably syrups, emulsions, and suspensions. Suitable carriers for syrups include saccharose or saccharose in admixture with glycerine and/or mannitol and/or sorbitol. Suitable carriers for suspensions and emulsions include natural gums, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. Suitable carriers for suspensions or solutions for intramuscular injections include preferably sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol. A suitable amount of lidocaine hydrochloride can optionally be contained in injectable preparations.

Suitable carriers solutions for intravenous injection or infusion are sterile water or sterile isotonic saline.

Suitable excipients for suppositories include cocoa butter, polyethylene

glycol, polyoxyethylene sorbitan fatty acid ester surfactants or lecithins.

The following examples illustrate the invention in greater detail.

Example 1

N-(5,6-Dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine

5,6-Dimethoxy-3,4-dihydro-1H-naphthalen-2-one (1.5 g, 7.5 mmol), prepared as described in *J. Med. Chem.* 1977, 20, 1111-1116, was dissolved in water (15 ml) and a solution of O-ethylhydroxylamine hydrochloride (1 g, 10 mmol) and Na_2CO_3 (0.53 g, 5 mmol) in water (10 ml) was added dropwise under stirring at 10°C. The reaction was left at room temperature overnight and then extracted with diethyl ether. The ether solution was evaporated to dryness under vacuum. The residue was dissolved in 20 ml of ethanol and concentrated hydrochloric acid (1 ml) and hydrogenated at 3,6 x 10^6 Pa (50 psi) using PtO_2 as catalyst. The solvent was removed under reduced pressure, water was added and the aqueous phase was treated with $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered and concentrated to dryness under vacuum. The crude residue was purified by chromatography, to afford 0.85 g of the title compound.

MS (EI): 251.0 (M^+).

Example 2

N-(5,6-Dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl- hydroxylamine

N-(5,6-Dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine (0.85 g, 3.4 mmol), obtained as described in Example 1, was dissolved in 2-pentanone (10 ml) and refluxed with 1-bromopropane (0.5 g, 4 mmol) and solid K_2CO_3 (0.6 g, 4.5 mmol). The solid was filtered and the solvent was evaporated to dryness under vacuum. The crude residue (1.2 g) was purified by chromatography to afford 0.28 g of the title compound.

MS (EI): 293.2 (M^+).

Example 3

N-(5,6-Dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine

N-(5,6-Dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine (0.8 g, 2.7 mmol), obtained as described in Example 2, was dissolved in 48% HBr (12 ml) and refluxed until completion of the reaction. The solvent was evaporated to dryness under vacuum and the residue was purified by chromatography (CH_2Cl_2/MeOH 90:10) to afford 0.5 g of the title compound.

MS (EI): 265.2 (M^+);

^{1}H-NMR (DMSO+TFA) δ: 6.62 (d, 1H); 6.41 (d, 1H); 4.07 (q, 2H); 3.50-3.62 (m, 1H); 3.18-3.27 (m, 2H); 2.70-3.01 (m, 3H); 2.18-2.30 (m, 1H); 1.61-1.76 (m, 3H); 1.15 (t, 3H); 0.92 (t, 3H).

The following compounds are obtained according to the same procedures described in examples 1-3:

- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-N-propyl-O-ethyl-hydroxylamine;

- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-O-methyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-O-ethyl-hydroxylamine;
- N-(5-hydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dihydroxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(5,6-dimethoxy-1,2,3,4-tetrahydro-2-naphthalenyl-methyl)-N-propyl-O-ethyl-hydroxylamine.

Example 4

N-(1-Methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-O-ethyl-hydroxylamine

1-(3,4-Dimethoxyphenyl)-2-propanone (1.35 g, 7.5 mmol) was dissolved in H_2O (15 ml) and a solution of O-ethylhydroxylamine hydrochloride (1 g, 10 mmol) and Na_2CO_3 (0.53 g, 5 mmol) in water (10 ml)

was added dropwise under stirring at 10°C. The reaction was left at room temperature overnight and then extracted with diethyl ether. After evaporation of the solvent, the residue was dissolved in EtOH (20 ml) and concentrated hydrochloric acid (1 ml), then hydrogenated over PtO_2 at 3,6 x 10^6 Pa (50 psi). The solvent was removed under vacuum. The residue was dissolved in 30 ml of water, the aqueous phase was made basic with $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was purified by flash chromatography, to afford 0.75 g of the title compound.

MS (EI): 239.3 (M^+).

Example 5

N-(1-Methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine

N-(1-Methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-O-ethyl-hydroxylamine (0.3 g, 1 mmol), obtained as described in Example 4, was dissolved in acetonitrile (10 ml) and refluxed with 1-bromopropane (0.135 g, 1.1 mmol) and solid K_2CO_3 (0.83 g, 6 mmol). The solid was filtered and the solvent was evaporated to dryness under vacuum. The crude residue (0.4 g) was purified by flash chromatography to afford 0.25 g of the title compound.

MS (EI): 281.3 (M^+).

Example 6

N-(1-Methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine

N-(1-Methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine 0.25 g, 0.9 mmol), obtained as described in Example 5, was dissolved in 48% HBr (4 ml) and refluxed until completion of the reaction. The solvent was evaporated to dryness under vacuum and the crude residue was purified by chromatography (CH_2Cl_2/MeOH 90:10) to afford 0.16 g of the

title compound.

MS (EI): 253.3 (M^+);

^{1}H-NMR (DMSO) δ: 6.62 (d, 1H); 6.56 (s, 1H); 6.41 (d, 1H); 3.73 (q, 2H); 2.92-3.08 (m, 1H); 2.80-2.88 (m, 1H); 2.61-2.73 (m, 2H); 2.18-2.28 (m, 1H); 1.45-2.58 (m, 2H); 1.08 (t, 3H); 0.86-0.95 (m, 6H).

Anal. ($C_{14}H_{23}NO_3 \cdot C_2HF_3O_2$) C, H, N and F.

The following compounds are obtained according to the same procedures described in examples 4-6:

- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-methyl-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3-hydroxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-O-methyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dihydroxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-methyl-hydroxylamine;

- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(1-methyl-2-(3-methoxy-phenyl)-ethyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-hydroxylamine;
- N-(1-methyl-2-(3,4-dimethoxy-phenyl)-ethyl)-N-propyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-methyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-propyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dihydroxy-phenyl)-propyl)-N-propyl-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-O-ethyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-methyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-propyl-hydroxylamine;
- N-(2-methyl-3-(3,4-dimethoxy-phenyl)-propyl)-N-propyl-O-ethyl-hydroxylamine.

Example 7

Inhibition of Aβ 1-42 spontaneous aggregation

Preparation of the Aβ 1-42 peptide

Synthetic Aβ 1-42 (U.S. Peptide, Rancho Cucamonga, USA) was dissolved to 220 μM in H_2O/CH_3CN 1:1. Aliquots of 10 μg were lyophilized under vacuum with an Eppendorf concentrator for 18 h and stored at -80°C.

Aβ 1-42 spontaneous aggregation

10 μg of lyophilized peptide sample was dissolved at 20 μM in 20 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl. The sample was incubated for 18 h at 37°C. After centrifugation at 13000 xg for 5 min, the pellet was dissolved in formic acid and both the pellet and the supernatant were analysed by HPLC. The extent of aggregation was determined as the percentage of peptide content in the pellet compared with the total amount.

HPLC analysis of the Aβ 1-42 peptide monomer

Column: PLRP-S 100 Å, 8 μm, 150 x 4.6 mm, Polymer Laboratories

Mobile phase: gradient from 15% A to 70% B in 10 min
A = H_2O + 0.01% TFA
B = CH_3CN + 0.08% TFA

Flow rate: 0.7 ml/min

Detector: UV, 214 nm

Example 8

Inhibition of Non Aβ Component of Alzheimer's Disease Amyloid (NAC, α-synuclein) spontaneous aggregation

Preparation of the NAC peptide

The synthetic peptide NAC (Bachem) was dissolved at 1 mg/ml in H_2O/CH_3CN 1:1 plus 5% TFA. Aliquots of 40 μg were lyophilized under vacuum for 18 h and stored at -80°C.

NAC spontaneous aggregation

40 μg of lyophilized peptide sample was dissolved at 500 μM in 20 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl. The sample

was incubated for 24 h at 37°C. After centrifugation at 13000 xg for 5 min, the pellet was dissolved in formic acid and both pellet and supernatant were analyzed by HPLC. The extent of aggregation was determined as the percentage of peptide content in the pellet compared to the total amount used.

HPLC analysis of the NAC peptide monomer

	1 pump
	1 autosampler
	1 UV detector
Guard column:	high performance guard column, 5 µm, Vydac
Column:	Protein and Peptide C18, 5 µm, 25 x 0.46 cm, Vydac
Mobile phase:	gradient developed from 95% A to 100% B in 12 min
	A = H_2O + 0.1% TFA
	B = CH_3CN + 0.08% TFA
Flow rate:	1 ml/min
Detector:	UV, 214 nm

Example 9

Inhibition of PrP 106-126 spontaneous aggregation

Preparation of the Prp 106-126 peptide

The synthetic peptide PrP 106-126 (Bachem) was dissolved at 1 mg/ml in H_2O/CH_3CN 1:1. Aliquots of 30 µg were lyophilized under vacuum for 18 h and stored at -80°C.

PrP 106-126 spontaneous aggregation

30 µg of lyophilized peptide sample was dissolved at 500 µM in 20 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl. The sample was incubated for 24 h at 37°C. After centrifugation at 13000 xg for 5 min, the pellet was dissolved in formic acid and both pellet and supernatant were analyzed by HPLC. The extent of aggregation was determined as the

percentage of peptide content in the pellet compared to the total amount used.

HPLC analysis of the PrP 106-126 peptide monomer

	1 pump
	1 autosampler
	1 UV detector
Guard column:	high performance guard column, 5 µm, Vydac
Column:	Protein and Peptide C18, 5 µm, 25 x 0.46 cm, Vydac
Mobile phase:	gradient developed from 95% A to 70% B in 12 min
	A = H_2O + 0.1% TFA
	B = CH_3CN + 0.08% TFA
Flow rate:	1 ml/min
Detector:	UV, 214 nm

Example 10

Thioflavine T (ThT) binding assay

After aggregation, the sample was centrifuged and the supernatant was discarded. The pellet was resuspended in 300 µl of 50 mM glycine-NaOH buffer, pH 9.4 containing 2 µM ThT and incubated for 5 min. The fluorescence was determined by a fluorescence plate reader (Fusion, Packard) at a 400 nm excitation wavelength and a 485 nm emission wavelength.